

WHAT IS CLAIMED IS:

- 5 1. A GBV-B replicon comprising the following regions:
a GBV-B 5' UTR substantially similar to bases 1-445 of SEQ ID NO
1;
a selection or reporter sequence functionally coupled to said GBV-B 5'
UTR;
an internal ribosome entry site;
10 a NS3-NS5B sequence substantially similar to bases 1938-7709 of
SEQ ID NO: 1 functionally coupled to said internal ribosome entry site and an AUG
translation initiation codon; and
a GBV-B 3' UTR substantially similar to bases 7710-8069 of SEQ ID
NO: 1,
15 wherein said replicon is capable of replication in a cell.
2. The GBV-B replicon of claim 1, further comprising a GBV-B
structural region, wherein said GBV-B structural region is functionally coupled to said
GBV-B 5' UTR.
- 20 3. The GBV-B replicon of claim 2, wherein
said GBV-B structural region comprises a sequence substantially
similar to a sequence selected from the group consisting of: bases 446-511 of SEQ ID
NO: 1, bases 446-487 of SEQ ID NO: 1, bases of 446-469 of SEQ ID NO: 1, the RNA
25 version of bases 446-2641 of SEQ ID NO: 2, and the RNA version of bases 446-3265
of SEQ ID NO: 2.
3. The GBV-B replicon of claim 3, wherein said replicon consists
of:
30 said GBV-B 5' UTR;
said GBV-B structural region;
said selection or reporter sequence;
said internal ribosome entry site;
said NS3-NS5B sequence; and
35 said GBV-B 3' UTR.

5. The GBV-B replicon of claim 4, wherein
said internal ribosome entry site has the sequence of bases 1324-1934
5 of SEQ ID NO 1;
said GBV-B structural region consisting of a sequence selected from
the group consisting of: bases 446-511 of SEQ ID NO: 1, bases 446-487 of SEQ ID
NO: 1, bases of 446-469 of SEQ ID NO 1, the RNA version of bases 446-2642 of
SEQ ID NO: 2 and the RNA version of bases 446-3265 of SEQ ID NO: 2;
10 said NS3-NS5B region is Met-NS3-NS5B region consisting of bases
1935-7709 of SEQ ID NO: 1; and
said GBV-B 3' UTR is bases 7710-8069 of SEQ ID NO: 1.
6. The GBV-B replicon of claim 5, wherein said GBV-B
15 structural region consists either of the RNA version of bases 446-2642 of SEQ ID
NO: 2 or the RNA version of bases 446-3265 of SEQ ID NO: 2.
7. The GBV-B replicon of claim 1, wherein said replicon consists
of SEQ ID NO: 1.
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8. The GBV-B replicon of claim 2, wherein
said GBV-B structural region comprises a sequence substantially
similar to a sequence selected from the group consisting of: bases 446-511 of SEQ ID
NO: 1, bases 446-487 of SEQ ID NO: 1, bases of 446-469 of SEQ ID NO: 1, and the
25 RNA version of bases 446-2641 of SEQ ID NO: 2.
9. The GBV-B replicon of claim 3, wherein said replicon consists
of:
said GBV-B 5' UTR;
30 said selection or reporter sequence;
said internal ribosome entry site;
said GBV-B structural region;
a NS2-NS5B region comprising a NS2 region substantially similar to
the RNA version of bases 2642-3265 of SEQ ID NO: 2 joined to the 5' end of said
35 NS3-NS5B region; and

said GBV-B 3' UTR.

10. The GBV-B replicon of claim 9, wherein
said internal ribosome entry site has the sequence of 1324-1934 of
5 SEQ ID NO 1;
said GBV-B structural region comprises a sequence selected from the
group consisting of: bases 446-511 of SEQ ID NO 1, bases 446-487 of SEQ ID NO 1,
bases of 446-469 of SEQ ID NO 1, and the RNA version of bases 446-2641 of SEQ
ID NO: 2;
10 said NS2-NS5B is a Met-NS2-NS5B region consisting of said 5' AUG
translation initiation codon, said NS2 region, and said NS3-NS5B region, wherein
said NS2 region consists of the RNA version of bases 2642-3265 of SEQ ID NO: 2
and said NS3-NS5B consists of bases 1938-7709 of SEQ ID NO: 1; and
said GBV-B 3' UTR is bases 7710-8069 of SEQ ID NO: 1.

- 15 11. The GBV-B replicon of claim 10, wherein said replicon
produces an infectious virion.

12. An expression vector comprising a promoter transcriptionally
20 coupled to a nucleotide sequence coding the GBV-B replicon of any one of claim 1-
11.

13. A GBV-B replicon made by a process comprising the steps of
transfecting a cell with the replicon of any one of claims 1-11 and isolating said
25 replicon.

14. The GBV-B replicon of claim 13, wherein said cell is either a
Huh7 cell, a Hep3B cell, is derived from a Huh7 cell, or is derived from a Hep3B cell.

- 30 15. A method of making a second GBV-B replicon from a first
GBV-B replicon comprising the steps of:
a) transfecting a cell with said first replicon, wherein said first replicon
is the replicon of any one of claims 1-11;
b) isolating a replicon from said transfected cell;

c) determining the nucleotide sequence of said replicon from said transfected cell; and

d) producing said second replicon, wherein said second replicon contains the first replicon sequence with one or more alterations corresponding to said replicon from said transfected cell.

16. The method of claim 15, wherein said cell is either a Huh7 cell, a Hep3B cell, is derived from a Huh7 cell, or is derived from a Hep3B cell.

17. A method of measuring the ability of a compound to affect GBV-B replicon activity comprising the steps of:

a) providing said compound to a cell containing the GBV-B replicon of any one of claims 1-11; and

b) measuring the ability of said compound to affect one or more replicon activities as a measure of the effect on GBV-B replicon activity.

18. The method of claim 17, wherein said cell is a human hepatoma cell.

19. The method of claim 18, wherein said cell is either a Huh7 cell, a Hep3B cell, is derived from a Huh7 cell, or is derived from a Hep3B cell.

20. A GBV-B replicon enhanced cell, wherein said cell has an maintenance and activity efficiency of at least 25% when transfected with a GBV-B replicon of SEQ ID NO: 1 by the Electroporation Method.

21. The cell of claim 20, wherein said cell is derived from a human hepatoma cell.

22. The cell of claim 21, wherein said cell is derived from a Huh7 cell or Hep3B cell.

23. The cell of claim 20, wherein said cell is a B76.1/Huh7 cell (ABC deposit PD02002) cured of its replicon.

24. A method of making a GBV-B replicon enhanced cell comprising the steps of:

- a) introducing and maintaining the GBV-B replicon of any one of claims 1-11 in a cell; and
- 5 b) curing said cell of said GBV-B replicon to produce said replicon enhanced cell.

25. The method of claim 24 further comprising

- c) introducing and maintaining a second GBV-B replicon in said
- 10 replicon enhanced cell, wherein said second replicon is genomic replicon; and
- d) curing said cell produced in step (c) of said genomic replicon.

26. The method of claim 24, wherein said cell is either a Huh7 cell, a Hep3B cell, is derived from a Huh7 cell, or is derived from a Hep3B cell.

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27. A method of making a GBV-B replicon enhanced cell containing a functional GBV-B replicon comprising the steps of:

- a) introducing and maintaining a first GBV-B replicon in a cell, wherein said first replicon is the replicon of any one of claims 1-11;
- 20 b) curing said cell of said first replicon to produce a cured cell; and
- c) introducing and maintaining a second GBV-B replicon into said cured cell, wherein said second GBV-B replicon may be the same or different than said first GBV-B replicon.

28. The method of claim 27, wherein said cell is either a Huh7 cell, a Hep3B cell, is derived from a Huh7 cell, or is derived from a Hep3B cell.

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29. The method of claim 28, wherein the GBV-B coding sequences in said first and second GBV-B replicons are the same.

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30. The method of claim 28, wherein said second replicon is a full-length replicon consisting of the RNA version of SEQ ID NO 2.

31. The method of claim 28, wherein said second replicon is a

35 replicon consisting of a first cistron and a second cistron, where said first cistron

comprises a GBV-B 5' UTR and the RNA version of bases 446-2642 of SEQ ID NO: 2 or the RNA version of bases 446-3265 of SEQ ID NO: 2; and said second cistron comprises an internal ribosome entry site functionally coupled to bases 1935-7709 of SEQ ID NO 1.

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32. A GBV-B replicon enhanced cell made by the method of claim 24.

10 33. A GBV-B replicon enhanced cell containing a GBV-B replicon comprising the cell of any one of claims 20-22 and either the replicon of any one of claims 1-11 or a full-length monocistronic GBV-B replicon.

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34. The GBV-B replicon enhanced cell containing a GBV-B replicon of claim 33, wherein said replicon is the replicon of any one of claims 1-11.

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35. The GBV-B replicon enhanced cell containing a GBV-B replicon of claim 33, wherein said replicon is said full-length monocistronic GBV-B replicon, and said full-length GBV-B replicon consists of the RNA version of SEQ ID NO: 2.

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36. A GBV-B replicon enhanced cell containing a GBV-B replicon made by the method of claim 27.

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37. A method of measuring the ability of a compound to affect GBV-B activity comprising the steps of:

- a) providing said compound to the GBV-B replicon enhanced cell containing a replicon of claim 33; and
- b) measuring the ability of said compound to affect one or more replicon activities as a measure of the effect on GBV-B activity.

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38. The method of claim 37, wherein said step (b) measures GBV-B protein production.

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39. The method of claim 37, wherein said step (b) measures production of RNA transcripts.

40. A method of measuring the ability of a compound to affect GBV-B activity comprising the steps of:

- 5 a) providing said compound to the GBV-B replicon enhanced cell containing a replicon of claim 36; and
b) measuring the ability of said compound to affect one or more replicon activities as a measure of the effect on GBV-B activity.

41. A method of producing an infectious GBV-B virion comprising
10 the step of culturing the replicon enhanced cell containing the replicon of claim 33 under conditions suitable for producing said GBV-B virion, wherein said replicon is said full-length monocistronic GBV-B replicon.

42. A method of infecting an animal with a GBV-B virion
15 comprising the steps of (a) producing said virion using the method of claim 41; and
(b) providing said virion to said animal.

43. The method of claim 42, wherein said animal is a tamarin or an owl monkey (*Aotus* species).
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44. A method of producing an infectious GBV-B virion comprising
the step of culturing the replicon enhanced cell containing a replicon of claim 36 to produce said GBV-B virion, wherein said second replicon is a full-length GBV-B replicon.
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45. A method of infecting an animal with a GBV-B virion
comprising the steps of (a) producing said virion using the method of claim 44; and
(b) providing said virion to said animal.

46. The method of claim 45, wherein said animal is a tamarin or an owl monkey (*Aotus* species).
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47. A method for producing a chimeric GBV-B/HCV replicon comprising the step of replacing one or more GBV-B regions or portion thereof

present in the replicon of any one of claims 1-11 with the corresponding region from HCV.